REMARKS

This Reply is responsive to the final Office Action dated September 7, 2007. Entry of the amendments, evidence and remarks submitted herein and reconsideration of the claimed subject matter is respectfully requested.

I. Status of the Claims

Claims 68-174 were pending in this application at the time of the Office Action dated September 7, 2007. Claims 68-106, 169 and 170 were withdrawn from consideration pursuant to a restriction requirement, but have not been cancelled. Claims 107-168 and 171-174 were under examination at the time of the Office Action dated September 7, 2007. Claim 107 has been amended to correct a grammatical error. Applicants submit that no prohibited new matter has been introduced by way of this amendment.

II. Priority Determination

Applicants acknowledge with appreciation the indication at page 2 of the Office Action that claim 139 has been accorded a priority date of 4/19/2000.

III. Rejection under 35 USC §103

Claims 107-168 and 171-174 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Werther *et al.* (US 5,929,040), Fire *et al.* (US 6,506,559), Heifetz *et al.* (WO 99/61631), Calabretta *et al.* (US 5,734,039) and Thompson *et al.* (US 6,146,886). Essentially, the Examiner has issued a new rejection of claim 174 and maintained the rejection of claims 107-168 and 171-173 under 35 USC §103 (a) for the

same reasons set forth in the Office Action dated March 8, 2007. According to the Office Action, Werther *et al.* allegedly teaches a multivalent antisense molecule but does not disclose the use of double stranded RNA sequences or the expression of double stranded RNA sequences from a vector. Calabretta *et al.* allegedly describes a composition comprising two antisense molecules directed to one or more target genes and suggests that the antisense molecules might be expressed from a single vector using two different promoters. However, as the Examiner acknowledges, Calabretta *et al.* does not teach the use or expression of double stranded RNA sequences and does not actually demonstrate expression of multiple RNA sequences. Nevertheless, the Examiner believes it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute double stranded RNA as allegedly disclosed in Fire *et al.* and Heifetz *et al.* as an alternative for the antisense sequences in the constructs of Werther and Calabretta and express these molecules in a vector as allegedly also disclosed in Fire *et al.* and Heifetz *et al.* Thompson is relied upon for teaching expression of therapeutic RNAs including ribozymes and antisense RNAs using a RNA pol III promoter. Applicants respectfully traverse the rejection.

The instant claims are directed to multitarget, partially double stranded RNA molecules comprising two or more different double stranded RNA sequences that are substantially homologous and complementary to two or more sequences of at least one target mammalian gene or mammalian pathogen gene (claim 107), vectors expressing such multitarget constructs (i.e., claim 141), and expression vectors encoding multiple double stranded RNA sequences that are expressed from different promoters (i.e., claim

150). None of the cited references alone or in combination render the subject matter of the instant claims obvious.

A skilled artisan would not have been motivated to substitute the double stranded RNA molecules disclosed in Fire and Heifetz for the antisense sequences in the constructs disclosed by Werther and Calabretta as the Examiner alleges. In this regard it is important to recognize that neither Fire nor Heifetz teaches the use of double stranded RNA molecules in mammalian cells. Heifetz, in fact, deals exclusively with plants, and Fire deals exclusively with nematodes. Although the Fire application suggests that double stranded RNA might be useful for inhibition of gene expression in mammals, it does not exemplify or enable such a use.

The distinction between the use of double stranded RNA in mammals versus plants and nematodes is not trivial. At the time the invention was made, an extensive body of literature existed which documented the induction of an interferon or PKR response when double stranded RNA molecules, particularly long double stranded RNA molecules, were introduced into mammalian cells. The PKR response results in a non-specific inhibition of gene expression due, in part, to the phosphorylation and inactivation of the eIF2 alpha translation factor by PKR kinase. This action by the PKR kinase shuts down general protein synthesis and triggers cell death. Thus, a skilled artisan attempting to silence specific mammalian target genes would not use the double stranded RNA molecule approach as he would expect a suppression of all protein synthesis and possible cell death as extensively described in the literature at that time. The declaration by Dr. McCallus submitted herewith supports the view point of a skilled artisan in the field at the time the invention was made.

Dr. McCallus holds a doctoral degree in immunology and has worked extensively in the field of RNA interference. He submits that those in the field, including himself, at the time the invention was made, believed that double stranded RNA molecules would induce an interferon response in mammalian cells and that this toxicity would prevent the successful use of double stranded RNA in inhibiting the gene expression of specific target genes in mammals (see paragraphs 3 and 4 of the Declaration by Dr. McCallus). Thus, the art effectively taught away from using double stranded RNA molecules as claimed in the present invention for sequence-specific gene silencing in mammalian cells.

Despite the references cited in Applicants' last reply documenting the occurrence of the PKR response in mammalian cells, the Examiner maintains her position that Fire teaches the use of double stranded RNA molecules for silencing gene expression and one would be motivated to construct a multitarget molecule as claimed in the present invention for use in mammals. The Examiner further states that it was known in the art that long double stranded RNA molecules could effectively silence gene expression because such molecules were cleaved into shorter double stranded RNA molecules by dicer. Applicants note again that Fire discloses methods of silencing gene expression with double stranded RNA molecules in nematodes, which do not exhibit an interferon or PKR response. Moreover, in contrast to what the Examiner asserts, it was not known at the time the present invention was made that longer double stranded molecules were cleaved by dicer. The Examiner has offered no authority for this statement.

As Dr. McCallus notes in his declaration, the PKR response was well documented and limited the use of RNAi applications in mammalian cells (see paragraph 4 of the declaration). The paper by Caplen *et al.*, which Dr. McCallus cites in his declaration,

emphasizes this point further by stating that "Numerous reports have described failures to observe gene-specific RNAi effects in different vertebrate systems, demonstrating instead nonspecific effects of dsRNA on gene expression. These nonspecific effects have not been surprising as there is an extensive literature describing a variety of non-specific responses induced by dsRNAs in mammalian cells." See Caplen *et al.* page 9742, col. 2. In any case, the implication by the Examiner that long double stranded RNA molecules would be effective in silencing expression of target genes because they would be cleaved into shorter double stranded RNA molecules by dicer and presumably avoid the PKR response is incorrect. Cleavage of the long double stranded RNA molecules by dicer is the initial step in the RNA interference pathway and would have no effect on the induction of the PKR response. As discussed above, activation of the PKR kinase would still suppress protein synthesis and trigger cell death, thus masking any potential effect of dicer-generated small interfering RNA molecules from longer double stranded molecules.

In view of Dr. McCallus' declaration and the references cited therein, it is clear that the skilled artisan would not have had a reasonable expectation of success of using long double stranded RNA molecules to inhibit specific target genes in mammalian cells. Therefore, it would not have been obvious to a skilled artisan to produce multitarget constructs comprising double stranded RNA molecules for use in mammalian cells. Given that the art taught away from using long double stranded RNA molecules and that there was no reasonable expectation of success, a conclusion of nonobviousness should be reached.

The present invention also claims expression vectors encoding multiple double stranded RNA sequences that are expressed from different promoters. This general

embodiment of the invention is also nonobvious over the cited references. The Examiner maintains that one of ordinary skill in the art would be motivated to make a single vector comprising two promoters for expressing different double stranded RNA molecules since Calabretta discloses such a vector for expressing different antisense molecules. She further argues that Heifetz et al. allegedly teaches expressing a sense and antisense sequence of a double stranded RNA from a single vector, and thus one of ordinary skill in the art would have a reasonable expectation of success to make a single vector encoding multiple double stranded RNA sequences expressed from different promoters as claimed in the instant application. Applicants respectfully disagree.

Contrary to the Examiner's assertion, the skilled artisan would not have been motivated to express different double stranded RNA sequences from separate promoters on a single vector due to the well known problem of promoter interference. Dr. McCallus notes in his declaration that the use of more than one promoter on a single vector typically resulted in preferential expression of a gene from one of the promoters and reduced or absent expression from the other promoter (see paragraph 6 of the declaration). He describes that the field devised a partial solution to the problem for obtaining efficient expression of protein products from a single vector, but that this solution was not workable if one were interested in obtaining RNA products (see paragraph 7). Dr. McCallus states that "promoter interference remained a recognized problem to the skilled artisan" and at the time the invention was made he, himself, was concerned that genes expressed from different promoters on a single vector would not be expressed with similar efficiencies (paragraphs 6 and 7).

Furthermore, Applicants note that the disclosure of Heifetz does not teach the expression of multiple double stranded RNA molecules from different promoters, but rather a sense and an antisense strand of a *single* double stranded RNA molecule in plants. Thus, one cannot infer from the Heifetz disclosure that a vector encoding *multiple* double stranded RNA molecules under the control of different promoters would be successful in mammals. In addition, Calabretta merely suggests the possibility of expressing the two different antisense sequences from different promoters on a single vector. In light of the literature describing the problems encountered with promoter interference, it is not apparent that a construct suggested by Calabretta would be successful or that the skilled artisan would have had any expectation of success with such a construct.

In summary, the skilled artisan would not have been motivated to produce long multitarget double stranded RNA molecules that target mammalian genes. First, the skilled artisan would not have attempted to use long double stranded RNA molecules to silence specific mammalian genes since the skilled artisan was aware of the PKR response induced by such RNA molecules in mammalian cells as well as the numerous failures by others to do so. Further, the skilled artisan would not have been motivated to express several different double stranded RNAs from a single vector, since it was well known in the prior art that competition between promoters (promoter interference) on a single vector resulted in unequal expression of the genes under the control of those promoters. Thompson was only relied upon for teaching expression of therapeutic RNAs including ribozymes and antisense RNAs using a RNA pol III promoter. Thompson does not make up for the deficiencies of Werther, Calabretta, Fire and Heifetz with regard to

teaching multitarget dsRNA molecules and vector constructs expressing multiple dsRNAs. In view of all the above remarks, reconsideration and withdrawal of the rejection under §103(a) are respectfully requested.

CONCLUSION

Applicants believe that this Reply adequately addresses all the rejections of the claims, and that the claims are now in condition for allowance.

Except for issue fees payable under 37 CFR §1.18, the commissioner is hereby authorized by this paper to charge any additional fees during the pendency of this application including fees due under 37 CFR §1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-1283. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 CFR §1.136(a)(3).

If the Examiner has any further questions relating to this Reply or to the application in general, she is respectfully requested to contact the undersigned by telephone so that allowance of the present application may be expedited.

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